CRISPR methods for product discovery and fungal cell factory construction

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Plenary session 6: System and synthetic biology

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12.30 – 13.00
Gaston Berger

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CRISPR methods for product discovery and fungal cell factory construction

The rapidly increasing number of fully sequenced fungal genomes constitutes a goldmine of new research possibilities. However, for most newly sequenced species, exploitation of this potential is hampered by low gene targeting frequencies. We have therefore developed a flexible CRISPR/Cas9 toolbox adapted for filamentous fungi to facilitate genome editing. Our toolbox currently contains a set of four AMA1 based vectors with different selections markers, which all contain a *cas9* gene codon optimized for *Aspergillus niger* and a USER cloning cassette for simple insertion of the sequences encoding *sgRNA*. Moreover, we have also developed an sgRNA design software that facilitates identification of *sgRNA* sequences that can target a desired gene in several different species, hence, reducing the plasmid construction workload. To investigate the versatility of our system we have embarked on a project aiming at testing tool parts in the black *Aspergillus* species where several species have not previously been genetically engineered. To address the efficiency of the system and to facilitate future work, we initially introduce mutations in homologs of the pigment gene *albA* and in the marker gene *pyrG*. In species where CRISPR-Cas9 works efficiently, we envision that it will now be possible to rapidly address specific scientific questions by changing or eliminating specific gene functions using our system directly; or by using it to eliminate the NHEJ pathway setting the stage for conventional gene targeting. We will provide examples of this strategy. CRISPR-Cas9 may also be advantageously used for cell factory construction as our versatile system allows genes to inserted and tested for activity in several different species using the same building blocks. In this way the repertoire of strains for production of a given product can be quickly tested and the best host chosen at an early point. For examining pathways in more detail or for metabolic engineering purposes, we are investigating the possibility of editing genes by RNA guided Cas9 activity using oligo-nucleotides as repair template for the repair of the resulting DNA DSBs. We have shown that it is possible to introduce defined point mutations in specific genes using this strategy in a number of genes. Efficiencies and possibilities will be discussed.